

UK Patent Application (19) GB (11) 2 251 186 (13) A

(43) Date of A publication 01.07.1992

(21) Application No 9026278.3

(22) Date of filing 04.12.1990

(71) Applicants

Randall Neal Gatz
Chemin de la Montau, CH-1261 Genolier, Vaud,
Switzerland

Peter Alfred Bromley
37 Chemin Cret-de-la-Neige, CH-1234 Vessy, Geneva,
Switzerland

(72) Inventors

Randall Neal Gatz
Peter Alfred Bromley

(74) Agent and/or Address for Service

Murgitroyd & Company
Mitchell House, 333 Bath Street, Glasgow, G2 4ER,
United Kingdom

(51) INT CL⁸

A61K 37/02 39/00

(52) UK CL (Edition K)

A5B BHA B170 B180 B31X B31Y B317 B823 B826
B829 B835 B838 B842
U1S S1332 S2416 S2419

(56) Documents cited

GB 2221157 A EP 0322990 A1 WO 88/10120 A1
WO 85/05034 A1
Chin.exp.Immunol. 1990, 81, 189-194
Autoimmunity 1990, 7, 237-244
Immunology 1969, 16(2), 157-165

(58) Field of search

UK CL (Edition K) A5B BHA
INT CL⁸ A61K 37/02 39/00
Online databases: WPI, DIALOG/PHARM

(54) Polypeptide for use in treatment of autoimmune disease

(57) The use of a polypeptide comprising an amino acid sequence not homologous to a sequence synthesised by the cells of the patient, for the manufacture of a medicament for the treatment of an autoimmune disease is described.

1 Autoimmune Disease Treatment

2

3 This invention relates to the treatment of autoimmune
4 diseases, and especially the prophylactic treatment of
5 such diseases.

6

7 Stress of a varied nature, induced as a result of heat
8 shock, nutrient deprivation, oxygen radicals and other
9 forms of metabolic disruption, including infection by
10 certain viruses, bacteria and protozoans, as well as
11 certain cases of cellular transformation, all lead to
12 the increased synthesis of a family of proteins
13 collectively known as stress proteins or heat shock
14 proteins.

15

16 These stress proteins are among the most highly
17 conserved and abundant proteins found in nature.
18 Further these proteins have been shown to be among the
19 dominant antigens recognised in immune responses to a
20 broad spectrum of pathogens. A review of the
21 interrelationships between stress proteins, infection
22 and immune surveillance has recently appeared, in
23 which a clear analysis of these relationships is
24 provided (13).

25

1 It has become apparent in recent years that a
2 relationship exists between so-called stress or heat
3 shock proteins and certain immune responses to
4 infection and to the development of autoimmunity. As
5 an example, the analysis of cell-mediated and humoral
6 responses to a variety of bacterial and parasitic
7 pathogens has shown that heat shock proteins are often
8 strongly immunogenic during infection (1-8).

9
10 Proteins involved in immune responses to certain
11 parasitic diseases such as malaria, shistosomiasis,
12 leishmaniasis, trypanosomiasis and filariasis, have
13 been identified as members of the hsp 70 and 90 gene
14 families. Further antigens related to hsp 70 and GroEL
15 families have been shown to play a role in T cell and
16 B cell recognition during bacterial infections
17 including leprosy, tuberculosis and Q. fever. The
18 mycobacterial GroEL stress protein has been identified
19 as the target of a T cell clone capable of causing
20 autoimmune disease in a rat model of adjuvant-induced
21 arthritis (9). Similar results have been obtained as
22 concerns the small heat shock proteins, since an
23 immunologically important 19 Kd protein antigen of
24 Mycobacterium leprae has been sequenced, and shown to
25 have considerable amino acid sequence homology to the
26 soybean 19Kd heat shock protein.

27
28 Elevated responses to the GroEL stress protein have
29 been found by testing T cells from synovial infiltrates
30 of rheumatoid arthritis patients (10). Autoantibodies
31 to hsp 90 have been reported in systemic lupus
32 erythematosus (SLE) (11). In addition, elevated
33 antibody responses to hsp70 and GroEL stress proteins
34 have been found in SLE and in rheumatoid arthritis
35 (12).

1
2 The stress proteins are remarkable in their
3 evolutionary conservation: hsp90, hsp70, and hsp60
4 proteins are found in all prokaryotes and eukaryotes.
5 In fact comparison of almost any two hsp70 proteins
6 from two different organisms indicates an amino acid
7 homology of around 50%. The major stress proteins
8 occur at low levels in normal, unstressed cells, but
9 accumulate to very high levels in cells undergoing
10 stress. A striking example is the case of E. coli
11 hsp60, which accounts for 1.6% of total cell protein
12 under normal growth conditions, and can accumulate to
13 15% of total cell protein after heat shock (14).
14 Stress proteins appear to fulfil vital roles in cells,
15 both in the absence and in the presence of stress.
16 They appear to be involved in the assembly and
17 disassembly of protein complexes, and hsp70 proteins
18 are important for the translocation of certain
19 proteins through cellular membranes (15). Stress
20 proteins appear to interact with many different
21 proteins, for example, hsp90 has been found to interact
22 with steroid hormone receptors and with viral and
23 cellular kinases. Hsp70 proteins bind to DNA
24 replication complexes, clathrin baskets, the cellular
25 tumour antigen p53, and immunoglobulin heavy chains.
26 Plant hsp60 interacts with Rubisco, which fixes CO₂ in
27 chloroplasts, and may be the most abundant protein in
28 the biosphere (16). The interaction of stress
29 proteins with multiple proteins may provide an
30 explication for the evolutionary constraints imposed
31 on their amino acid sequences.
32
33 Stress proteins have an almost certain role in
34 protecting cells and organisms from the deleterious
35 effects of heat and other stresses.

1
2 It seems clear that the tight sequence regulation
3 imposed on many heat shock protein sequences throughout
4 evolution has led to such retained sequences between
5 those of the host and those of the infectious agent
6 having a significant degree of identity. Clearly the
7 reaction of the host immune system against antigens of
8 the infecting organism could lead to the raising of
9 antibodies against heat shock proteins. The sequence
10 homology within the heat shock protein family thus
11 points to conserved sub-sequences of heat shock
12 proteins as being serious candidates for inducing an
13 immune response that can have specificity against self
14 sequences, with the consequence of inducing an
15 autoimmune reaction and the associated disease states.

16
17 The reports referenced above indicate that stress
18 proteins, such as the heat shock proteins, provide
19 particularly attractive targets for immune recognition.
20 An analysis the cross reactivity of T cell responses to
21 stress proteins has been published recently (17),
22 wherein the presence of human T cells was demonstrated
23 that were capable of immune recognition of conserved
24 sequence determinants. These authors have proposed a
25 model in which immune responses to stress proteins
26 provide a link between infectious and autoimmune
27 diseases.

28
29 Although models of the role of stress proteins in
30 autoimmune diseases have been proposed, no-one has yet
31 suggested possible treatment for autoimmune diseases.

32
33 In accordance with a first aspect of the present
34 invention a method of treating an autoimmune disease in
35 a patient comprises introducing a compound, comprising

1 an amino acid sequence of a protein which is not
2 homologous with amino acid sequences synthesised by
3 cells of the patient, into the patient.
4

5 In accordance with another aspect of the present
6 invention there is provided use of a compound
7 comprising an amino acid sequence of a protein for the
8 treatment of an autoimmune disease in a patient,
9 wherein the amino acid sequence is not homologous with
10 amino acid sequences synthesised by cells of the
11 patient.
12

13 Further, the invention provides a composition for
14 treatment of an autoimmune disease in a patient,
15 comprising a compound which comprises an amino acid
16 sequence of a protein which is not homologous with
17 amino acid sequences synthesised by cells of the
18 patient, in combination with a pharmaceutical carrier.
19

20 Still further, the invention provides the use of a
21 compound comprising an amino acid sequence of a protein
22 which is not homologous with amino acid sequences
23 synthesised by the cells of a patient for the
24 manufacture of a medicament for the treatment of an
25 autoimmune disease in the patient.
26

27 Preferably, the compound comprises a peptide which
28 comprises the amino acid sequence and typically, the
29 protein is a stress or heat shock protein.
30

31 Preferably, the treatment is prophylactic.
32

33 Typically, the compound could be introduced into a
34 patient by incorporation in a cream or ointment, in a
35 soluble glass, in slow release capsules, transdermal

1 patches, injected, or even administered orally or in
2 suppository form.

3
4 Preferably, the amino acid sequence has antigenic
5 properties.

6
7 The amino acid sequence could be naturally occurring or
8 be synthesised. If the amino acid sequence is
9 synthesised then the peptide could comprise a number of
10 different amino acid sequences and/or multiples of the
11 same amino acid sequence.

12
13 The invention described here is based on the
14 above-detailed conservation of heat shock sequences and
15 their implication in autoimmune diseases. Contrary to
16 the identity of certain conserved sequences, this
17 invention, is based on the hypervariable sequences of
18 stress proteins. Prior immunisation with natural or
19 synthetic peptides representing such non-conserved,
20 variable or hypervariable stress protein sequences of
21 origin from infectious agents of bacterial and other
22 parasitic pathogens, induces antibody responses
23 against the stress proteins of the infecting organism,
24 and these specifically induced antibodies are incapable
25 of recognising self stress protein sequences. The
26 rapid recognition of infectious agent - specific stress
27 proteins by specific pre-existing antibodies raised
28 against non-homologous peptides from invading stress
29 proteins should allow the elimination of these stress
30 proteins before they are able to elicit potentially
31 autoimmune responses.

32
33 This invention concerns the immune recognition of
34 peptide epitopes of specific heat shock or stress
35 proteins, and the development of peptide-based therapy

1 or prevention based on such epitopes.

2

3 Examples of the invention will now be described.

4

5 1. Analysis of stress protein peptide sequences

6

7 In order to practice the preventive/therapeutic
8 approach described in this invention, it is necessary
9 to examine in detail the amino acid sequences of human
10 heat shock proteins, and of those of organisms
11 infecting human beings with whom correlations of immune
12 diseases exist.

13

14 Our initial approach was to assemble a table of certain
15 of the known sequences of stress proteins from human
16 and infectious agent sources. A selection of these
17 sequences are presented in Appendix 1. A thorough
18 analysis of sequence homology between members of each
19 of the stress protein families indicates that for
20 each of the principle stress protein families, hsp70,
21 hsp90 and hsp27, certain sequences have been highly
22 conserved throughout evolution, whereas parts of the
23 stress proteins contain amino acid sequences that are
24 highly differentiated. One assumes that the
25 conservational pressures concerning the retained
26 sequences are associated with critical structural or
27 functional aspects of these important proteins. The
28 variable regions are presumably of less critical
29 structural or functional importance, thus escaping
30 from the conservative pressure/selection activities
31 prevailing in evolving organisms.

32

33

34 2. Selection of candidate peptide vaccines

35

1 The selection of useful candidate peptides capable of
2 eliciting an immune response specifically against the
3 stress proteins of the infectious agent is based on
4 two major criteria:

5
6 i) The non-identity of selected peptide sequences,
7 and their lack of resemblance to highly, or partially
8 conserved stress protein sequences, common to human
9 and infectious agent proteins. The selection of such
10 non-conserved sequences is derived from a reverse
11 analysis of amino acid sequence homologies, in other
12 words, concentrating on the non-homologous sequences
13 evident from homology analyses such as those shown in
14 (1) and in appendix 2.

15
16 For a thorough selection of sequence differences
17 versus sequence homology, it is instructive to, in
18 addition to amino acid identity, to look at
19 replacements by highly conserved amino acids. Examples
20 of such substitutions are the following groups:
21 (aspartic acid and glutamic acid), (lysine and
22 arginine), (serine and threonine), (phenylalanine and
23 tyrosine), and (isoleucine, leucine, valine and
24 methionine).

25
26 ii) An analysis of the antigenic potential of selected
27 peptide sequences. Where information is available,
28 peptide epitopes that conform to the criteria of both
29 points i) and ii), and which can be demonstrated to be
30 immunodominant, are preferred examples of the
31 preventive/therapeutic peptides described in this
32 invention.

33
34 Examples of the amino acid sequences of some selected
35 peptides that reply to the criteria of point i) are

1 presented in appendix 2.

2

3 Examples of group i) peptides that are expected to
4 have considerable immunogenic potential have been
5 selected on the basis of presently accepted criteria
6 of immunological potential. Examples of certain
7 peptides with pronounced antigenicity are shown in
8 appendix 3.

9

10 Non-homologous sequence comparison of the known stress
11 protein and related antigen sequences from humans and
12 from infectious agents has been performed. In the case
13 of Plasmodium falciparum, in addition to regions of
14 extensive homology of amino acid sequence between the
15 two proteins, clear regions of extensive lack of
16 homology are also detectable, and the following
17 sequence fragments, depicted using the one and
18 three-letter amino acid abbreviations derived from the
19 IUPAC-IUB Commission on Biochemical Nomenclature (see
20 Table 1), illustrate this example:-

21

22 ALIGNMENT OF RESIDUES 133 TO 254 OF 75KDa antigen of P
23 Falciparum TO RESIDUES 357 TO 635 OF HSP70 HUMAN

24

25 ENYCYGVKSSLEDKIKEKLP AEIETCMKTITTTILEWLEKNQLAGKDEYE

26 ----- KNALES-Y-AFNMKSA- VEDEG LKGKIS-E

27

28 AKQKEAESVQAPIMSKIY-QDAA-GAAGGMPGGM-P-GGMPGGMP GGMNF

29 ADKKKVLDKQCQEVIS- WLDANTLA EKDEFEHKRKELEQVCNPIISGL-Y

30

31 PG-GMPG-AGMPGNAP---AGSGPTVEEVV

32 QGAGGPGPGGFGAQQGPKGGSGSGPT-----

33

34 Examples of non-homologous peptides are shown in bold
35 letters. The second peptide of HSP70 human shown in

1 bold above, denoted "Peptide example 1", has been
 2 compared to the sequence of the corresponding antigen
 3 of Mycobacterium tuberculosis and its highly unique
 4 sequence has little or no counterpart in the sequence
 5 of tubercular origin.

6
 7 ALIGNMENT OF RESIDUES 8 TO 11 OF PEPTIDE 1 TO RESIDUES
 8 1 TO 127 OF 71KDa antigen M.tuberculosis
 9

10 K----R--K-- E-----
 11 KEDIDRMKDAEAAEEDRKRREEADVNRGAETLVYNTEKFVKEQREGG
 12

13 Clearly other peptide sequences unique to an infectious
 14 agent antigen exist and will have value in the
 15 applications described in this invention. In order to
 16 identify such sequences, extensive cloning, expression
 17 and sequence analysis of infectious agent antigens
 18 will be required. Such research, although technically
 19 arduous, is quite within the realms of existing
 20 technology. Similarly, once new sequences are
 21 established, the presence or absence of amino acid
 22 sequence homologies can be determined either
 23 visually, or through the use of any number of amateur
 24 or commercial sequence analysis software programs. Our
 25 intention here is to demonstrate the general procedure
 26 for identifying, and applying both specific
 27 non-homologous and specific homologous stress and
 28 infectious agent antigen peptide sequences to the
 29 vaccination, therapeutic and cosmetic applications
 30 described herein.

31
 32
 33
 34
 35

3 The Rational Design of Synthetic Peptides

1 This invention is not limited to naturally occurring
2 variant sequences within stress proteins, nor is it
3 limited to the selection and use of a single variant
4 epitope. For example, synthetic peptides could be
5 used. In addition, the peptide could be synthesised to
6 have combinations of different variant sequences or
7 multiples of variant sequences. By synthesising
8 peptides comprising different variant sequences and/or
9 multiples of the same variant sequence it may be
10 possible to design peptides having a stronger immune
11 response against stress proteins of infectious
12 organisms but which do not recognise human stress
13 epitopes.

14
15 A recent analysis of variant peptide epitopes of
16 myelin basis protein (MBP), and their influence on the
17 incidence of experimental autoimmune encephalomyelitis
18 (EAE) has indicated that synthetic variants of an
19 N-terminal MBP peptide can have greatly altered
20 properties of binding to cell surface glycoproteins
21 encoded by the major histocompatibility complex (MHC)
22 (18). In other words, the efficacy of the complex
23 interactions associated with the elicitation of an
24 effective immune response against peptide antigens, can
25 be altered and improved in some cases, by the use of
26 synthetic variants of natural antigens. The subject
27 of this invention comprises those variant peptide
28 sequence approaches that are taught by the authors
29 of reference 18, amongst others.

30
31 An efficient mapping procedure for identifying protein
32 antigenic determinants has been described that would
33 be of use in the selection of useful antigenic
34 determinants for the applications taught in this
35 invention (19). Clearly classical chemical, enzymatic

1 and combined synthetic procedures can be utilised to
2 produce candidate peptides, once identified and
3 selected, for the vaccine applications described here.
4 A naturally expected limitation of the peptide vaccines
5 that can be produced using this described procedure
6 derives from the fact that about one third of
7 monoclonal and polyclonal antibodies made by
8 immunising with native protein react with assembled
9 topographic sites (20). These assembled determinants
10 may not form the appropriate structure outside of a
11 proteins native environment. This limitation is not
12 expected to significantly limit the practical use of
13 this invention.

14
15 Studies concerning T Cell recognition and activation
16 have indicated that it may be possible to design
17 peptides with predictable and advantageous properties
18 (21). These authors have described two approaches for
19 immunomodulation that could be useful for the design
20 of therapeutic strategies against autoimmune
21 encephalomyelitis. The first approach consists of a
22 thorough molecular characterisation of an
23 encephalitogenic epitope, and the subsequent design of
24 peptide analogs that retain normal or increased major
25 histocompatibility complex binding properties, and that
26 fail to activate disease-inducing T cells. Secondly,
27 novel properties of a heterocyclic peptide have been
28 described, with the result that the peptide is highly
29 antigenic in vitro, while being non-immunogenic in
30 vivo. These authors have been able to demonstrate the
31 feasibility of immune intervention in an immune disease
32 through the use of a synthetic peptide. These results
33 are complementary to the procedure we describe here,
34 but are not identical, nor do they in any way predict
35 the approach that we describe.

1
2 4 Applications of the stress protein peptides described
3 herein
4

5 The basic tenant that we have developed herein is based
6 on the multiple observations that certain infectious
7 agent antigens are closely related in amino acid
8 sequence to human stress proteins, and that immune
9 reactions against such antigens can cross react with
10 the human proteins, leading to the possibility of
11 developing autoimmune disease. Our invention describes
12 the selection of stress protein peptide sequences from
13 infectious agent antigens related to human stress
14 proteins, but which have little or no sequence homology
15 within such human stress proteins. The injection of
16 such non-homologous peptides into human beings, for
17 instance in an emulsification with Freund's complete
18 adjuvant, would provide a route of effective
19 vaccination against subsequent autoimmune disease
20 induced as mentioned above. The antibodies raised
21 through such vaccination are specific to the selected
22 infectious agent antigen from which the vaccinating
23 peptide was derived. Such induced antibodies are
24 specific to infectious agent antigens, thus explaining
25 their efficacy in the application of this invention.
26

27 Further, since the vaccinating agent is a small
28 peptide, instead of a large, complex protein such as
29 human factor VIII, or factor IX, it is not compulsory
30 to use an injection as a means of delivering the
31 peptide to a human subject. We thus reserve in our
32 application the administration of the kinds of peptides
33 described by transdermal applications, a number of
34 which are presently commercialised with considerable
35 success.

1
2 Further still, since certain major diseases that are
3 thought to have their origin in autoimmune diseases,
4 such as arthritis and rheumatism, the peptides of this
5 invention can be applied externally, in both local and
6 cosmetic application to painful joints and
7 articulations resulting from these prevalent diseases.
8

9 For example, the peptides could be administered to a
10 patient by incorporation in a cream or ointment, in a
11 soluable glass, in slow release capsules, transdermal
12 patches, injected, or even administered orally or in
13 suppository form.
14

15 In addition, due to the nature of amino acid sequences
16 it is unlikely that treatment using these substances
17 will produce the unpleasant side effects which are
18 normally associates with drugs.
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35

APPENDIX 1

NON-HOMOLOGOUS SEQUENCES WHICH ARE ALSO
 KNOWN ANTIGENICS ARE DENOTED BY
 UNDERLINING AND NON-HOMOLOGOUS ONLY
 SEQUENCES ARE DENOTED BY BOLD LETTERING

SEQUENCE OF HUMAN STRESS PROTEINS

A) Sequence HSP90 Human

Rebbe N F, Ware J, Bertina M, Modrich P, Stafford D W
 Gene 53:235-245(1987)
 EMBL; M16660; HSHSP90
 KW Heat Shock. Sequence 724 AA; 83293 MW

21	MPEEVHHGEE	EVETFAFAQE	IAQLMSLIIN	TFYSNKEIFL	40
22	RELISNASDA	LDKIRYESLT	DPSKLDGSGE	LKIDIIPNPQ	80
23	ERTLTLDVDTG	IGMTKADLIN	NLGTIAKSGT	KAFMEALQAG	120
24	ADISMIGQFG	VGFYSAYLVA	EKVVVIRKHN	DDEQYAWESS	160
25	AGGSFTVRAD	HGEPIGMGTK	VILHLKEDQT	EYLEERRVKE	200
26	VVKKHSQFIG	YPITLYLEKE	REKEISDDEA	EEKGEKEEEE	240
27	DKDDEEKPKI	EDVGSDEEDD	SGDKKKKKTK	KIKEKYIDQE	280
28	ELNKTPIWT	RNPDDITQEE	YGEFYKSLTN	DWEDHLAVKH	320
29	FSVEGQLEFR	ALLFIPRRAP	FDLFENKKKK	NNIKLYVRRV	360
30	FIMDSCDELI	PEYLNFIKRV	VDSIDLPLNI	SREMLQQSKI	400
31	LKVIRKNIVK	KCLELFSELA	EDKENYKKFY	EAFSKNLKLG	440
32	IHEDSTNRRR	LSLLRYHTS	QSGDEMTSL	EYVSRMKETQ	480
33	KSIYYITGES	KEQVANSFV	ERVRKRGFEV	VYMTEPIDEY	520
34	CVQQLKEFDG	KSLVSVTKEG	LELPEDEEEK	KKMEESKAKF	560
35	ENLCKLMKEI	LDKKVEKVTI	SNRLVSSPCC	IVTSTYGWTA	600

1	NMERIMKAQA	LRDNSTMGYM	MAKKHLEINP	DHPIVETLRQ	640
2	KAEADKNDKA	VKDLVLLFE	TALLSSGFSL	EDPQTHSNRI	680
3	TYMIKLGLGI	DEDEVAAEEP	NAAVPDEIPP	LEGDEDASRM	720
4	EEVD				724
5					
6					
7					
8	B) Sequence HSP70 Human				
9					
10	[1] Hunt C, Morimoto R I;				
11	Proc Natl Acad Sci USA 82:6455-6459(1985)				
12	EMBL; M11236; HSHSP701				
13	EMBL; MII717; HSHSP70D				
14	KW Heat Shock				
15	Sequence 640AA; 69867 MW				
16					
17	MAKAAAVGID	LGTTYSCVGV	FQHGKVEIIA	NDQGNRTTPS	40
18	YVAFTDTERL	IGDAAKNQVA	LNPQNTVFDA	KRLIGRKFGD	80
19	PVVQSDMKHW	PFQVINDGDK	PKVQVSYKGE	TKAFYPEEIS	120
20	SMVLTKMKEI	AEAYLGYPVT	NAVITVPAYF	NDSQRQATKD	160
21	AGVIAGLNVL	RIINEPTAAA	IAYGLDRTGK	GERNVLIFDL	200
22	GGGTFDVSIL	TIDDGIFEVK	ATAGDTHLGG	EDFDNRLVNH	240 (3)
23	FVEEFKRKHK	KDISQNKRAV	RRLRTACERF	EGIDFYTSIT	280
24	RARFEELAKR	<u>TLSSSTOASL</u>	EIDSLCSDLF	RSTLEPVEKA	320 (4)
25	LRDAKLDKAQ	IHDLVLVGGS	TRIPKVQKLL	QDFFNGRDLN	360
26	KSINPDEAVG	YGAAVQAAIL	MGDKSENVQD	LLLLDVAPLS	400
27	LGLETAGGVM	TALIKRNSTI	PTKQTQIFTT	YSDNQPGVLI	440
28	QVYEGERAMT	KDNNLLGRFE	LSGIPPAPGV	PQIEVTFDID	480 (1)
29	ANGILNVTAT	DKSTGKANKI	TITNDKGRLS	KEEIERMVQE	520
30	AEKYKAEDV	<u>QREVSARKNA</u>	<u>LESYAFNMKS</u>	AVEDEGLK GK	560 (2)
31	ISEADKKKVL	DKCQEVISWL	DANTLAEKDE	FEHKRKELEQ	600
32	VCNPIISGLY	QGAGGPGPGG	FGAQGPKGGS	GSGPTIEEVD	640
33					
34					
35					

1 C) Sequence Human HSP27

2

3 Hickey E, Brandon S E, Potter R, Stein G, Stein J,
4 Weber L A;

5 Nucl. Acids Res 14:4127-4145(1986)

6 EMBL;X03900; HSHSP27

7 KW: HEAT SHOCK

8 SEQUENCE 199 AA; 22327 MW;

9

10	MTERRVPFSL	LRGPSWDPFR	DWYPHSRLFD	QAFGLPRLPE	40
11	EWSQWLGGSS	WPGYVRPLPP	AAIESPAVAA	PAYSRALSRQ	80
12	LSSGVSEIRH	TADRWRVSLD	VNHFAPDELT	VKTKDGVVEI	120
13	TGKHEERQDE	HGYISRCFTR	KYTLPPGVDP	TQVSSSLSP	160
14	GTLTVEAPMP	KLATQSNEIT	IPVTFESRAQ	LGGRSCKIR	200

15

16 D) Sequence Human HSP60

17

18 Sequence not yet available, submitted for publication:

19 Gupta R S, Jinal S, Harley C B and Dudani A K(1989)

20

21

22 SEQUENCE OF HSP60 YEAST

23

24

25 Reading D S, Hallberg R L and Myers A M (1989). Nature
26 337 655

27

28	MLRSSVVRSR	ATLRPLLRRR	YSSHKILKFG	VIGRASLLKG	40
29	VETLAIIVAA	TLGPKGRNVL	IEQPFPPKI	TKDGVTVAKS	80
30	IVLKDKFINM	GAKLLQIVAS	KTNIAAGDGT	TSATVLGRAI	120
31	FTISVKNVAA	GCPNMDLRRG	SQVAVIKVIL	FLSANKKEIT	160
32	TSEEIAQVAT	ISANGDSHVG	KLLASAMEKV	GKEGVITIRE	200
33	GRITLEDELE	VTEGMRFDGR	FISPYFITDP	KSSKVEFEKP	240
34	LLLLSEKKIS	SIQDILPALE	ISNQSRRLPL	IIAEDVDGEA	280
35	LAACILNKLK	GQVKVCAVKA	PGFGDNRKNT	IGDIAVLTGG	320

1	TVFTEELDLK	PEQCTIENLG	SCDSITVTKE	DTVILNGSGP	360
2	KEAIQERIEQ	IKGSIDITTT	NSYEKEKLQE	RLAKLSGGVA	400
3	VIRVGGASEV	EVGEKKDRYD	DALNATRAAV	EEGILPGGGT	440
4	ALVKASRVLD	EVVVDNFDQK	LGVDIIRKAI	TRPAKQIIEN	480
5	AGEEGSVIIG	KLIDEYGDDF	AKGYDASKSE	YTDMLATGII	520
6	DPFKVVRSGL	VDASGVASLL	ATTEVAIVDA	PEPPAAAGAG	560
7	GMPGGMPG	MPGMM			600

8

9

SEQUENCES OF BACTERIAL ANTIGENS

10

11

12 A) Mycobacterium leprae

13

14 18 KDa Antigen

15

16 Nerland A H, Mustapha A S, Sweetser D, Godal T, Young R

17 J Bacteriol 170 5919-5921 (1988)

18 Sequence 148 AA; 16643MW;

19

20 MLMRTDPFRE LDRFAEQVLG TSARPAVMPM DAWREGEEFV 40

21 VGFDLPGKA DSLDIDIERD VVTVRAERPG VDPDREMLAA 79

22 ERPRGVFNQ LVLGENLDTE RILASYQEGV LKLSIPVAER 119

23 AKPRKISVDR GNNGHQTINK TPHEIIDA

24

25

26 65 KDa Antigen

27

28

29 Mehra V, Sweetser D and Young R A (1986) Proc Natl Acad

30 Sci USA 83 7013

31

32 AA 589, MW 61,831

33 The Underling Amino Acids Correspond To Antigenic

34 Peptides.

35

1	VPGRDGETQP	ASCGRPSRAL	HPASVSNGGC	RSPVILASFL	40
2	IRRNHFAMAK	TIAYDEEARR	GLERGLNSLA	DAVKVTLGPK	80
3	<u>GRNVVLEKKW</u>	<u>GAPTITNDGV</u>	<u>SIAKEIELED</u>	PYEKIGAELV	120
4	KEVAKKTDDV	AGDGTTTATV	LAQALVKEGL	<u>RNVAAGANPL</u>	160
5	<u>GLKRGIEKAV</u>	DKVTETLLKD	AKEVETKEQI	AATAAISAGD	200
6	QSIGDLIAEA	MDKVGNEGVI	TVEESNTFG	LQLELTEGMR	240
7	FDKGYISGYF	VIDAERQEAV	LEEPYILLVS	SKVSTVKDLL	280
8	PLLEKVIQAG	KSLIIIAEDV	EGEALSTLVV	NKIRGTFKSV	320
9	AVKAPGFGDR	RKAMLQDMAI	LTGAQVISEE	VGLTLENTDL	360
10	SLLGKARKVV	MTKDETTIVE	GAGDTDAIAG	RVAQIRTEIE	400
11	NSDSYDREK	LQERLAKLAG	GVAVIKAGAA	TEVELKERKH	440
12	REIDAVRNAK	AAVEEGIVAG	GGVTLLQAAP	<u>ALDKLKLTDG</u>	480
13	<u>EATGANIVKV</u>	ALEAPLKQIA	FNSGMEPGVV	AEKVRNLSVG	520
14	HGLNAATGEY	<u>EDLLKAGVAD</u>	<u>PVKVTRSALO</u>	<u>NAASIAGLFL</u>	560
15	TTEAVVADKP	EKTAAPASDP	<u>TGGMGGMDF</u>		600

16

17

18 70 KDa Antigen

19

20

21 Not yet sequenced. Immunological cross-reactivity with
 22 the 71 KDa antigen of Mycobacterium tuberculosis (YOUNG
 23 ET AL Proc Natl Acad Sci USA 85, 4267-4270 (1988).

24

25

26 B) Mycobacterium tuberculosis

27

28

29 65 KDa Antigen

30

31

32 Schinnick, T S (1987). Journal of Bacteriology 169

33 1080

34 AA 562, MW 59083

35

1	RGCRHPVTPP	VSSPIRRNHV	AMAKTIAYDE	EARRGLERGL	40
2	NALADAVKVT	LGPGRNVVL	EKKWGAPTIT	NDGVSIKEI	80
3	ELETPYEKIG	AELVKEVAKK	TDDVAGDGTT	TATVLAQALV	120
4	REGLRNVAAG	ANPLGLKRG	EKAVEAKVTET	LLKGAKEVET	160
5	KEQIAATAAI	SAGDQSIGDL	IAEAMDKVGN	EGVITVEESN	200
6	TFGLQLELTE	GMRFDKGYIS	GYFVTDPERQ	EAVLEDPYIL	240
7	LVSSKVSTVK	DLLPLLEKVI	GAGKPLLIIA	EDVEGEALST	280
8	LVVNKIRGTF	KSAVKAPGF	GDRRKAMLQD	MAILTGGQVI	320
9	SEEVGLTLEN	ADLSLLGKAR	KVVVTKDETT	IVEGAGDTDA	360
10	IAGRVAQIRQ	EIENSDDSDYD	REKLQERLAK	LAGGVAVIKA	400
11	GAATEVELKE	RKHRIEDAVR	NAKAAVEEGI	VAGGGVTLLK	440
12	AAPTLDLKL	EGDEATGANI	VKVALEAPLK	QIAFNNGLEP	480
13	GVVAEKVRNL	PAGHGLNAQT	GVYEDLLAAG	VADPVKVTRS	520
14	ALQNAASAIG	LFLTTEAVVA	DKPEKEKASV	PGGGDMGGMD	560
15	F				600

16

17

18 71 KDa Antigen

19

20

21 Partial sequence, contains only the homolgy domain with
 22 HSP70

23

24 Young D, Lathigra R, Hendrix R, Sweetser D, Young R,

25 Proc Acad Sci

26 USA 85, 4265-4270 (1988).

27

28	EFQPSVQIQV	YQGEREIAAH	NKLLGSFELT	GIPPAPRGIP	40 (1)
29	QIEVTFDIDA	NGIVHVTAKD	KGTGKENTIR	IQEGSGLSKE	80
30	DIDRMKDAE	<u>AHAEEDRKRR</u>	<u>EEADVNRGAE</u>	TLVYNTEKFV	120 3,4
31	KEQREGGSKV	PEDTWRIGYF	GHQVGDDGEAG	PGVAGSGASD	160 (2)
32	LRSSSGCVTG	HWRCPPRAAA	GRCPPRLGM		200

33

34

35

1 C) Plasmodium falciparum (MALARIA)

2

3

4 90 KDa Antigen

5

6

7 M Jendoubi, S Bonnefoy, Nucl Acids Res 16, 10928 (1988)
8 Partial sequence, contains only the region of homology
9 with HSP90

10

11	KDFDGGKKLKC	CTKEGLDIHH	SEEAKKDFET	VIKDV LHKKV	40
12	EKVVCQRIT	DSPCVLVTSE	FGWSANMERI	MKAQALRDNS	80
13	MTSYMLSKKI	MEINARHP II	SALKQKADAD	KSDKTVKYLI	120
14	WLLFDTSLLT	SGFFALEEPT	TFSKRIHRMI	KLGLSIDE EE	160
15	NNDIDLPPLE	ETV DATDSKM	EEVD		200

16

17

18 75 KDa Antigen

19

20

21 Ardeshir F, Flint J E, Richman S and Reese R T, Embo J.
22 6, 493-499
23 (1987).

24 Partial sequence from the first AA

25

26	MLKLIERN TT	IPAKKSQIFT	TYADNQPGVL	IQVYEGERAL	40
27	TKDNNLLGKF	HLDGIPFAPR	KVPQIEVTFD	IDANGILDVT	80
28	AVEKSTGKQN	HITITNDKGR	LSQDEIDRMV	NDAEKYLAED	120
29	EENRKRIEAR	NSLENYCYGV	KSSLEDKIKE	KLQPAEIETC	160
30	MKTITTILEW	LEKNQLAGKD	EYEAKQKEAE	SVCAPIMSKI	200
31	YQDAAGAAGG	MPGGMPGGMP	GGMPGGMNFP	GGMPGAGMPG	240
32	NAPAGSGPTV	EEVVD			280

33

34

35

APPENDIX 2

DIFFERENTIATION OF HOMOLOGOUS (UNDERLINE)
AND NON-HOMOLOGOUS SEQUENCES

A) Alignment of Residues 47 to 161 of partial sequence
of P. Falciparum 90KD to residues 581 to 699 of human
HSP90

--RI-DSPCVLVTSEFGWSANMERIMKAQALRDNSMTSYMLSKKIMEINAR
NRLVSSPCCIVTSTYGWTANMERIMKAQALRDNSTMGYMAKKHLEINPD

HPIISALKOKADADKSDKTVKYLIWLLFDTSLLTSGFFALEEPTTFSKRI
HPIVETLRQAEADKNDKAVKDLVLLFETALLSSG-FSLEDPQTHSNRI

HRMIKLGLSIDEEE---NN
YRMIKLGLGIDEDEVAAEE

B) Alignment of residues 7 to 157 of partial sequence
of P. falciparum 70 KDa to residues 411 to 613 of human
HSP70.

-----NTTIPAKKSOIFTTYADNOPGVLIQVYEGERALTKDNNLLGKFHL
ALIKRNSTIPTKQTQIFTTYSNQPVGVLQVYEGERAMTKDNNLLGRFEL

DGIPPAPRKVPOIEVTFDIDANGILDVTAVEKSTGKONHITITNDKGRLS
SGIPPAP-GVPQIEVTFDIDANGILNVTATDKSTGKANKITITNDKDRLS

QDEIDRMVNDAEKYLAEDEENRKRIEARNLENYCYGVKSSLEDK-IKEKLO
KEEIERMVQEAKEYKADEVQRERSAKNALESYAFNMKSAVEDEGLKKGIS

PAETCMK---TITILEWLEKNOLAGKDEYEAKQKEAESVCAPIMSKIYQDA
EADKKKVLDKCQEVI-SWLDANTLAEKDEFEHKRKELEQVCNPIISGLYQGA

1
2 C) Alignment of residues 5 to 110 of M tuberculosis 71K
3 to residues 430 to 548 of human HSP70
4

5 -----VQIQVYQGEREIAAHNKLLGSFELTGIPPAPRGIPQIEVTFDI
6 YSDNQPGVLIQVYEGERAMTKDNNLLGRFELSGIPPAP-GVPQIEVTFDI
7

8 DANGIVHVTAKDKGTGKENTIRIQEGSG-LSKEDIDRMIKDAEAHAEDR
9 DANGILNVTATDKSTGKANKITITNDKGRLSKEEIERMVQEAKEYKAED
10

11 KRREEADV RNGAE-----

12 VQRERVSAKNALESYAFNM
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35

APPENDIX 3

Antigenic Peptides of the 65 Kda Antigen of
Mycobacterium leprae

MEHRA V, SWEETSER D and YOUNG R A (1986) Proc Natl Acad
Sci USA 83 7013

-NSLADAVKVTLGPKGRNVVLEKKWGAPTITNDGVS

-RNVAAGANPLGLKRGIEKAV

-ALDKLKLTGDEATGA

-GEYEDLLKAGVADP

-ASDPTGGMGGMDF

TABLE 1

One and Three Letter Amino Acid Abbreviations

1			
2			
3			
4			
5			
6	A	Ala	Alanine
7	C	Cys	Cysteine
8	D	Asp	Aspartic acid
9	E	Glu	Glumatic acid
10	F	Phe	Phenylalanine
11	G	Gly	Glycine
12	H	His	Histidine
13	I	Ile	Isoleucine
14	K	Lys	Lysine
15	L	Leu	Leucine
16	M	Met	Methionine
17	N	Asn	Asparagine
18	P	Pro	Proline
19	Q	Gln	Glutamine
20	R	Arg	Arginine
21	S	Ser	Serine
22	T	The	Threonine
23	V	Val	Valine
24	W	Trp	Tryptophane
25	Y	Tyr	Tyrosine
26	B	Asx	Asp or Asn (not
27			distinguished)
28	Z	Glx	Glu or Gln (not
29			distinguished)
30	X	X	Undetermined or atypical
31			amino acid
32			

33 From: IUPAC-IUB Commission on Biochemical
 34 Nomenclature, J Biol
 35 Chem 243, 3557-3559, 1968.

References

- 1 1 Young D.B, Lathigra R, Hendrix R, Sweetser D and
2 Young R A 1988. Stress proteins are immune targets in
3 leprosy and tuberculosis. Proc Natl Acad Sci USA 85
4 4267.
5
6
7
8 2 Vodkin M H and Williams J C 1988. A heat shock
9 operon in *Coxiella burnetti* produces a major antigen
10 homologous to a protein in both mycobacteria and
11 *Escherichia coli*. J Bacteriol 170 1227.
12
13 3 Bianco A E, Favaloro J M, Burkot T R, Culvenor J
14 G, Crewther P E, Brown G V, Anders R F, Coppel R L and
15 Kemp D J 1986. A repetitive antigen of *Plasmodium*
16 *falciparum* that is homologous to heat shock protein 70
17 of *Drosophila melanogaster*. Proc Natl Acad Sci USA 83
18 8713.
19
20 4 Ardesshir F, Flint J E, Richman S J and Reese R T
21 1987. A 75Kd merozoite surface protein of *Plasmodium*
22 *falciparum* which is related to the 70 Kd heat-shock
23 proteins. EMBO J 6 493.
24
25 5 Hedstrom R, Culpepper J, Harrison R A, Agabian N
26 and Newport G 1987. A major immunogen in *Schistosoma*
27 *mansoni* infections is homologous to the heat-shock
28 protein Hsp 70. J Exp Med 165 1430.
29
30 6 Selkirk M E, Rutherford P J, Denham D A, Partano F
31 and Maizels R M 1987. Cloned antigen genes of *Brugia*
32 *filarial* parasites. Biochem Soc Symp 53 91.
33
34 7 Dragon E A, Sias S R, Kato E A and Gabe J D 1987.
35 The genome of *Trypanosoma cruzi* contains a

- 1 constitutively expressed tandemly arranged multicopy
- 2 gene homologous to a major heat shock protein. Mol
- 3 Cell Biol 7 1271.
- 4
- 5 8 Jendoub M and Bonneloy S 1988. Identification of
- 6 a heat shock-like antigen in *P. falciparum* related to
- 7 the heat shock protein 90 family. Nucleic Acids Res 16
- 8 10928.
- 9
- 10 9 van Eden W, Thole J E R, van der Zee R, Noordzy A,
- 11 van Embden J D A, Hensen E J and Cohen I R 1988.
- 12 Coning of the mycobacterial epitope recognised by T
- 13 lymphocytes in adjuvant arthritis. Nature 331 171.
- 14
- 15 10 Res P C M, Schaar C G, Breedveld F C, van Eden W,
- 16 van Embden J D A, Cohen I R and de Vries R R P 1988.
- 17 Synovial fluid T cell reactivity against 65 Kd heat
- 18 shock protein of mycobacteria in early chronic
- 19 arthritis. Lancet 478.
- 20
- 21 11 Minota S, Koyasu S, Yahara and Winfield J 1988.
- 22 Autoantibodies to the heat shock protein hsp90 in
- 23 systemic lupus erythrematosus. J Clin Invest 81 106.
- 24
- 25 12 Tsoulfa G, Rook G A W, van Embden J D A, Young D
- 26 B, Mehlert A, Isenberg D A, Hay F C and Lydyard P M
- 27 1989. Raised serum IgG and IgA antibodies to
- 28 mycobacterial antigens in rheumatoid arthritis. Annals
- 29 of Rheumatic Diseases. 48 118.
- 30
- 31 13 Young R A and Elliot T J 1989. Stress Proteins,
- 32 Infection, and Immune Surveillance. Cell 59 5
- 33
- 34 14 Herendeen S L, van Bogelen R A and Neidhardt F C
- 35 1979. J Bacteriol 139 185.

- 1
- 2 15 Cheng M Y, Hartl F -U, Martin J, Pollock R A,
- 3 Kalousek F, Neupert W, Hallberg E M, Hallberg R L and
- 4 Horwich A L 1989. Nature 337 620.
- 5
- 6 16 Hemmingsen S M, Woolford C, van der Vies S M,
- 7 Tilly K, Dennis D T, Georgopoulos C P, Hendrix R W and
- 8 Ellis R J 1988. Nature 333 330.
- 9
- 10 17 Lamb J R, Bal V, Mendez-Samperio P, Mehlert A, So
- 11 A, Rothbard J, Jindal S, Young R A and Young D B 1989.
- 12 Stress proteins may provide a link between the immune
- 13 response to infection and autoimmunity. The Japanese
- 14 Society for Immunology 0953 8178/89, International
- 15 Immunology Vol 1 No 2.
- 16
- 17 18 Urban J L, Horvath S J and Hood L 1989.
- 18 Autoimmune Recognition of Normal and Variant Peptide
- 19 Epitopes and Peptide-Based Therapy. Cell 59 257.
- 20
- 21 19 Mehra V, Sweetser D and Young R A 1986. Efficient
- 22 mapping of protein antigenic determinants. Proc Natl
- 23 Acad Sci USA 83 7013.
- 24
- 25 20 Berzofsky J A 1985. Science 229 932
- 26
- 27 21 Wraith D C, Smilek D E, Mitchell D J, Steinman L
- 28 and McDevitt H O 1989. Cell 59 247.
- 29
- 30
- 31
- 32
- 33
- 34
- 35

CLAIMS

- 1
2
3
4
5 1. A method of treating an autoimmune disease in a
6 patient comprises introducing a compound,
7 comprising an amino acid sequence of a protein
8 which is not homologous with amino acid sequences
9 synthesised by cells of the patient, into the
10 patient.
11
- 12 2. Use of a compound comprising an amino acid
13 sequence of a protein for the treatment of an
14 autoimmune disease in a patient, wherein the amino
15 acid sequence is not homologous with amino acid
16 sequences synthesised by cells of the patient.
17
- 18 3. A composition for treatment of an autoimmune
19 disease in a patient, comprising a compound which
20 comprises an amino acid sequence of a protein
21 which is not homologous with amino acid sequences
22 synthesised by cells of the patient, in
23 combination with a pharmaceutical carrier.
24
- 25 4. The use of a compound comprising an amino acid
26 sequence of a protein which is not homologous with
27 amino acid sequences synthesised by the cells of a
28 patient for the manufacture of a medicament for
29 the treatment of an autoimmune disease in the
30 patient.
31
32
33
34
35

Patents Act 1977
Examiner's report to the Comptroller under
Section 17 (The Search Report)

Application number

9026278.3

Relevant Technical fields

Search Examiner

UK CI (Edition K) A5B(BHA)

C SHERRINGTON

UK CI (Edition 5) A61K 39/00, 37/02

Databases (see over)

Date of Search

UK Patent Office

3 FEBRUARY 1992

ONLINE DATABASES:WPI, DIALOG/PHARM

Documents considered relevant following a search in respect of claims

Category (see over)	Identity of document and relevant passages	Relevant to claim(s)
X	GB 2221157 A (BIOGAL GYOGYSZERGYAR) especially page 1, line 12-14; Claim 19	4
X	EP 0322990 A1 (DE STAAT DER NEDERLANDEN...) whole document	4
X	WO 88/10120 A1 (BRIGHAM AND WOMEN'S HOSPITAL) whole document especially page 6, line 19 - page 7, line 3; Example 6; Claims 1-10, 13-19	4
A	WO 85/05034 A1 (UNIVERSITY OF LONDON ET AL) especially page 2, line 18 - page 3, line 4; Claims 3-5	4
X	Clin.exp.Immunol.1990,81,189-194 Prevention of adjuvant arthritis in rats by a nonapeptide from the 65-kd...	4
X	Autoimmunity 1990,7,237-244 The immune response to Mycobacterial heat shock proteins	4
X	Immunology 1969,16(2),157-165 Inhibition of Adjuvant Arthritis by Protein Antigens	4

Category	Identity of document and relevant passages	Relevant to claim(s)

Categories of documents

X: Document indicating lack of novelty or of inventive step.

Y: Document indicating lack of inventive step if combined with one or more other documents of the same category.

A: Document indicating technological background and/or state of the art.

P: Document published on or after the declared priority date but before the filing date of the present application.

E: Patent document published on or after, but with priority date earlier than, the filing date of the present application.

&: Member of the same patent family, corresponding document.

Databases: The UK Patent Office database comprises classified collections of GB, EP, WO and US patent specifications as outlined periodically in the Official Journal (Patents). The on-line databases considered for search are also listed periodically in the Official Journal (Patents).